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Research Report

Fish oil modulates glycogen synthase kinase-3 signaling pathway in diabetes-induced hippocampal neurons apoptosis

Li-Juan Sun[a,1], *Xiang-Hong Hou*[b,1], *Sen-Hai Xue*[a], *Feng Yan*[a], *Yu-Jie Dai*[a], *Chang-Hai Zhao*[a,*], *Feng Wang*[b,**], *Rui-Hua Yang*[b,***]

[a]*Department of Clinical Nutrition, Xi Jing Hospital, the Fourth Military Medical University, Xi'an 710032, PR China*
[b]*Department of Nutrition and Food Hygiene, the Ministry of Education Key Lab of Hazard Assessment and Control in Special Operational Environment, School of Public Health, the Fourth Military Medical University, Xi'an 710032, PR China*

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ABSTRACT

Previous research has demonstrated that diabetes induces learning and memory deficits. However, the mechanism of memory impairment induced by diabetes is poorly understood. Dietary fatty acids, especially polyunsaturated fatty acids, have been shown to enhance learning and memory and prevent memory deficits in various experimental conditions. The present study investigated the effects of fish oil supplementation on the neuron apoptosis in the hippocampus of streptozotocin (STZ)-induced diabetes rats, further explored the effect of fish oil on the phosphorylation of protein kinase B and glycogen synthase kinase-3 beta. The effects of diabetes and fish oil treatment on the spatial learning and memory were also evaluated using the Morris Water Maze. STZ-induced diabetes impaired spatial learning and memory of rats, which was associated with the apoptosis of hippocampal neurons and oxidative stress. Fish oil administration ameliorated cognitive deficit, reduced oxidative stress, increased AKT phosphorylation, decreased GSK-3β phosphorylation, and decreased pro-apoptotic molecules expression, which protected the hippocampal neurons from apoptosis in diabetic rats. These results suggested a potential role for fish oil as an adjuvant therapy for the prevention and treatment of diabetic complications.

Abbreviations: STZ, streptozotocin; DM, diabetes mellitus; FO, fish oil;PUFAs, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PI3K, phosphoinositide 3-kinase; AKT, Protein Kinase B (PKB); GSK-3β, glycogen synthase kinase-3β

*Co-Corresponding author at: Department of Clinical Nutrition, Xi Jing Hospital, The Fourth Military Medical University, Xián 710032, PR China.
**Co-Corresponding author at: Department of Nutrition and Food Hygiene, School of Public Health, The Fourth Military Medical University, Xián 710032, PR China.
***Corresponding author. Fax: 86 29 84774407.
E-mail addresses: 306078869@qq.com (C.-H. Zhao), wfeng@fmmu.edu.cn (F. Wang), fmmur.young@gmail.com (R.-H. Yang).
[1]These authors contributed equally to this work.

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1. Introduction

Diabetes mellitus (DM) is a common metabolic disease in human beings with characteristic symptoms of hyperglycemia, chronic inflammation (Leinonen et al., 2004; Martins et al., 2010) and insulin resistance. There is a substantial amount of experimental and clinical evidence that DM induced cognitive impairments, which have been partly associated with the structural and functional deficits in hippocampus (Brismar et al., 2007; Stranahan et al., 2008; Ye et al., 2011; Heng et al., 2011). In the previous studies, we found that diabetes induced apoptosis of hippocampal neurons, which contributed to the deficit of learning and memory of diabetic rats (Ye et al., 2011). However, the mechanism of neuron apoptosis in diabetic rats is not been fully elucidated. Phosphoinositide 3-kinase/Protein Kinase B (PI3K/AKT) signaling pathway is important in sustaining the survival and the function of neurons (Duarte et al., 2008). It has been reported that the dysfunction of PI3K/AKT signaling pathway increased glycogen synthase kinase-3β (GSK-3β) activity and led to tau protein hyperphosphorylation (Schubert et al., 2003), which was responsible for neurofibrillary tangles in the brain and have a positive relation with cognitive dysfunction.

Dietary fatty acids, especially n-3 polyunsaturated fatty acids (n-3 PUFA), have been shown to enhance learning and memory and prevent memory deficits in various experimental conditions. Docosahexaenoic acid (DHA, C22: 6 n-3), one of the major omega-3 polyunsaturated fatty acids in the brain, is important for brain development and plasticity, and provides support to learning and memory events in animal models of Alzheimer's disease (Hashimoto et al., 2002; Lim et al., 2005) and brain injury (Wu et al., 2004). DHA can affect neural function by enhancing long-term potentiation (McGahon et al., 1999). However, there was also some research which demonstrated the lacking of direct evidence on the effect of n-3 PUFA supplementation on cognitive function of human (Gould et al., 2013; Sydenham et al., 2012; Mazereeuw et al., 2012). Most of the positive results were obtained from animal experiments, but it is debatable whether or not fish oil are benefit for cognitive decline in human randomized controlled trials because of the difficulty in condition control, complex confounding factors, treatment duration, etc. However, it is beneficial to explore the effect and mechnisms of n-3 PUFA as one of adjuvant therapies. In light of these, we examined whether supplementation with fish oil (FO), a rich source of n-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5 n−3) and DHA, could attenuate the injury of hippocampal neurons of STZ-induced diabetic rats, and further explore the mechanisms.

2. Results

2.1. *Fish oil did not reduce the blood glucose levels of diabetic rats*

Streptozotocin injection resulted in a diabetic syndrome verified by the presence of polydypsia, polyuria, hyperglycemia, and weight loss in the diabetic animals. Mean blood glucose levels in both of the diabetic groups were significantly higher than the control group ($p<0.01$) after STZ injection, By the end of week 5, blood glucose levels in both diabetic groups remained significantly elevated (Fig. 1A). Significant weight loss was observed in STZ-induced diabetic rats compared with the control group (Fig. 1B, $p<0.01$). To demonstrate the insulin sensitivity, we performed oral glucose tolerance test. Area under the curve (AUC) of glucose responsed to oral glucose solution were higher in diabetic rats (Fig. 1D, $p<0.01$). Compare to the diabetic group, the blood glucose at 60 min after glucose administration significantly lower in fish oil treated diabetic rats (Fig. 1C, $p<0.05$). However, there was not significant difference between AUC of diabetic and diabetic+FO groups (Fig. 1D). There were no significant difference of both blood glucose and body weight between standard chow and fish oil supplement diet.

2.2. *Fish oil reduced the neuronal apoptosis induced by diabetes*

The apoptotic cells were detected by TUNEL stain in the hippocampus slices. In control and fish oil groups, TUNEL-positive neuron was absent in the hippocampus (Fig. 2A and C). In contrast, significant amounts of TUNEL-positive neurons appeared in the hippocampal CA1 area in diabetic rats (Fig. 2B,E). TUNEL-positive cells were also found in the fish oil treated diabetic rats (Fig. 2D). However, when compared with the diabetic group, fish oil treatment significantly decreased the total number of TUNEL-positive cells (Fig. 2E).

2.3. *Effect of fish oil on the expression of apoptotic related molecules*

In order to explore the effect of fish oil on anti-apoptotic and pro-apoptotic molecules expression, we examined B-cell leukemia-2 (Bcl-2), Bcl-2-associated X protein (Bax), and cytochrome-c (Cyt-c) on hippocampal CA1 area. Immunohistochemistry labeling demonstrated that anti-apoptic Bcl-2 protein was expressed moderately in all of three groups (Fig. 3A). The number of Bax and Cyt-c positive neurons increased significantly in diabetic group (Fig. 3C, E2). The number of Bax and Cyt-c positive neurons declined in fish oil treated diabetic rats (Fig. 3C, E3). The statistic analysis of difference between different groups was showed in Fig. 3D and F.

Levels of Bcl-2, Bax, and Cyt-c in the hippocampus were further detected by Western blot and quantitatively analyzed (Fig. 4). Correspondingly, Bax, and Cyt-c immunoreactivity was increased markedly in diabetic group compared with the control group, and fish oil administration reversed the effect of diabetes, although the expression did not reach control level. There were no significant changes of Bcl-2 immunoreactivity in all of the three groups.

The expression of cleaved caspase-3 and caspase-9 in the hippocampus are shown in Fig. 5. Caspase-3 and caspase-9 immunoreactivity increased markedly in diabetic animals compared to the control group (Fig. 5). Fish oil treatment antagonized the over expression of caspase-3 and caspase-9